Agilent 6410 Triple Quad LC/MS System

Quick Start Guide

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Use this guide for your first steps with the Agilent 6410 Triple Quad LC/MS, and as a roadmap for your user information.

What is the Agilent 6410 Triple Quad LC/MS system?

The Agilent Triple Quad LC/MS is a liquid chromatograph-triple quadrupole mass spectrometer that performs MS/MS using three sets of parallel rods (in this case, quadrupole, hexapole, quadrupole). The first quadrupole separates ions into precursor ions that are fragmented in the hexapole into product ions, which are separated by the second quadrupole. Often, two or more precursor ions and their product ions are monitored in sequence in MRM (multiple reaction monitoring) mode.

You can set up an Agilent Triple Quad LC/MS with either the Agilent 1100LC or 1200LC modules and one of several ion sources, ESI, APCI, APPI, or Multimode. You can also use the HPLC-Chip Cube with the Agilent Triple Quad LC/MS. With the ESI source, the instrument shows a sensitivity in the low femtograms.

Also, the Agilent Triple Quad LC/MS comes with Agilent MassHunter Workstation Software that includes three programs:

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- Data Acquisition – From one screen you can tune the mass spectrometer, control and monitor instrument parameters, set up acquisition methods and worklists containing multiple samples and monitor real-time run plots.
- Quantitative Analysis – From one screen you can set up a batch of data files and quantify, evaluate and requantify the results. From this screen you have access to the Method Editor for setting up and editing the quantitation methods.
- Qualitative Analysis – From one screen you can extract and integrate chromatograms, subtract background, extract peak spectra, and compare data from different types of data files.

What's New in Data Acquisition B.01.03

The Data Acquisition program for 6410 Triple Quad has many new features in this revision.

- G6410A-2K is supported with a mass range up to 2000 amu.
- APPI source is supported.
- HPLC-Chip is supported.
- Nano pump and capillary pump are now supported.
- The µWPS is now supported.
- You can now display instrument curves in the Real-time Plot window.
- You can now autotune when the source is set to ESI, MMI or HPLC-Chip.
- When the MassHunter Data Acquisition Compliance Software is installed, the Acquisition program identifies the user logged on. Different users have different defined capabilities. Only users in some groups will have permission to edit the method.

For information on what is new in the Qualitative Analysis and Quantitative Analysis programs, refer to the *Familiarization Guide* for each of these programs.

Where to find information

Help

Press F1 To get more information about a pane, window or dialog box, place the cursor on the part of the pane, window or dialog box of interest and press **F1**.

Help menu From the Help menu, access “How-to” help and reference help.

Documents

You can find these manuals delivered with the Triple Quad hardware or software. You can also find a PDF version on the installation disk, in the **Manuals** folder.

Installation Guide This guide is used to install the hardware and software, configure the instrument, and verify performance.

Concepts Guide - The Big Picture Learn the background information to help you understand operation of the hardware and software.

Maintenance Guide Use this guide to help maintain and troubleshoot your Agilent Triple Quad LC/MS.

Data Acquisition for 6410 Triple Quad Familiarization Guide Do the exercises to learn to use the Triple Quad LC/MS hardware and Data Acquisition program for 6410 Triple Quad.

Qualitative Analysis Familiarization Guide Do the exercises to learn to use the Qualitative Analysis program. *(This guide is on the Qualitative Analysis disk.)*

Quantitative Analysis Familiarization Guide Do the exercises to learn to use the Quantitative Analysis program. *(This guide is on the Quantitative Analysis disk.)*

Training

Familiarization Guide Use all three guides to get to know the software.

Training Courses Visit www.chem.agilent.com to view a listing of training courses for the Agilent Triple Quad LC/MS.

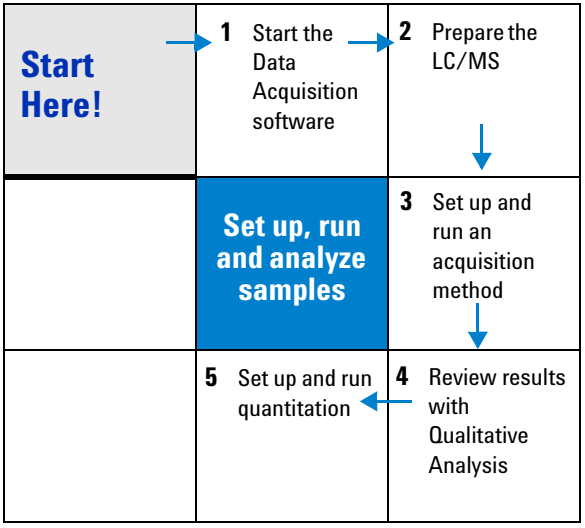
Getting Started

Install the Triple Quad LC/MS hardware and software

Use the *Installation Guide* to install the hardware and software and verify performance.

Set up, run and analyze samples

The roadmap below shows you the steps to set up and run a batch of samples from start to finish. Follow the instructions on the next pages to get started and to learn where to find the information to help you with each step in this roadmap.


Start Here!
1 Start the Data Acquisition software
2 Prepare the LC/MS
Set up, run and analyze samples
3 Set up and run an acquisition method
5 Set up and run quantitation
4 Review results with Qualitative Analysis

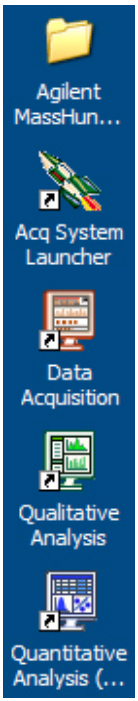
Step 1. Start the Data Acquisition software

The instructions below include the following assumptions:

- The hardware and software are installed.
- The instrument is configured.

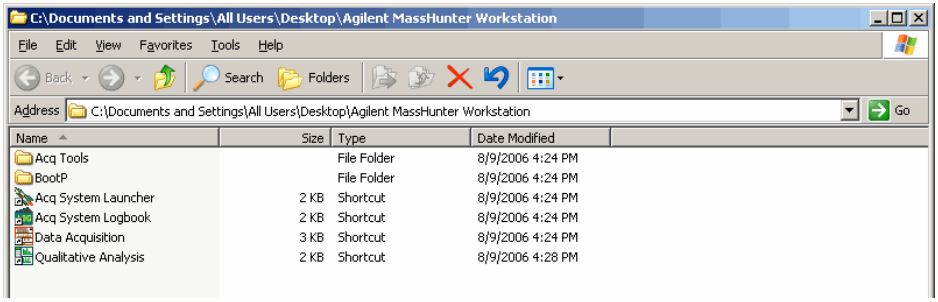
  Use instructions in the *Installation Guide* to configure the instrument for the first time and any time you change the LC configuration.
- The LC modules and the Triple Quad MS are turned on, but the LC pump is not running.

After installation, you see all of the Agilent MassHunter Workstation Software icons on your Desktop.



- To start the Data Acquisition program, double-click the **Data Acquisition** icon.

  The Data Acquisition window appears.


C:\Documents and Settings\All Users\Desktop\Agilent MassHunter Workstation
File Edit View Favorites Tools Help
Back Search Folders
Address C:\Documents and Settings\All Users\Desktop\Agilent MassHunter Workstation
Go
Name Size Type Date Modified
Acq Tools File Folder 8/9/2006 4:24 PM
BootP File Folder 8/9/2006 4:24 PM
Acq System Launcher 2 KB Shortcut 8/9/2006 4:24 PM
Acq System Logbook 2 KB Shortcut 8/9/2006 4:24 PM
Data Acquisition 3 KB Shortcut 8/9/2006 4:24 PM
Qualitative Analysis 2 KB Shortcut 8/9/2006 4:28 PM

NOTE

When Data Acquisition opens, the software engines automatically start. If for any reason, they are stopped and you need to restart them, double-click the **Acq System Launcher** icon on your Desktop, and click **Start**.

If you have recently changed LC modules, remember to configure the instrument again. See the *Installation Guide* for instructions.

Four panes—where you do most of your work

When you first start the Data Acquisition program, the main window appears. You do almost all of your work within the five panes of this main window. These panes provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status, monitor runs and tune the instrument.

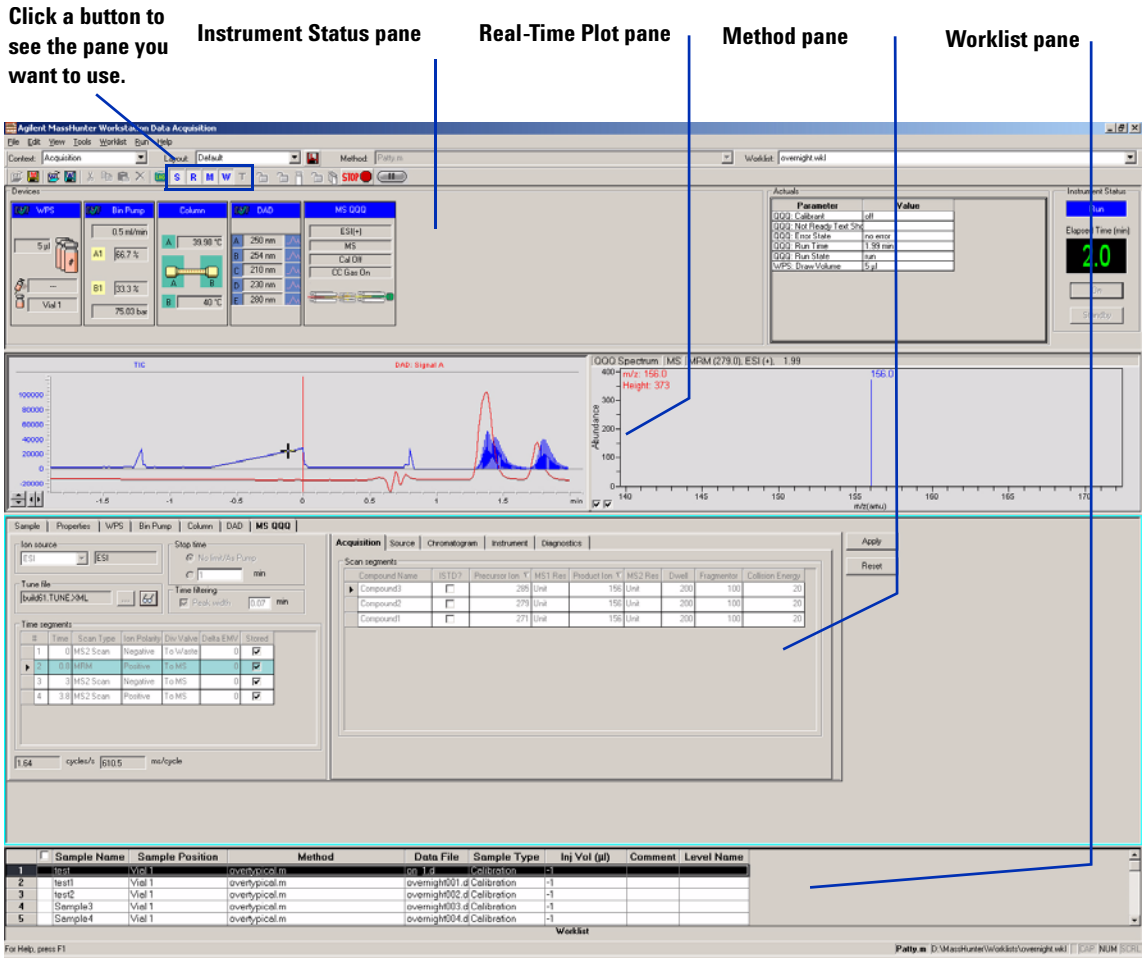


Figure 1 Main window of the Data Acquisition software component

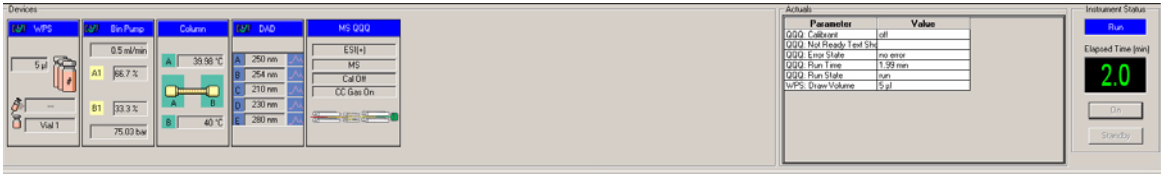
Show/hide the panes You can show one pane at a time on the screen or up to four panes. You can never hide all four panes. To show or hide a pane, you click the icons in the main window toolbar or you use the commands in the **View** menu.

When you click on a pane, the active pane is outlined in blue. Press F1 to obtain help on the active pane. You can also drag a pane border to resize the pane.

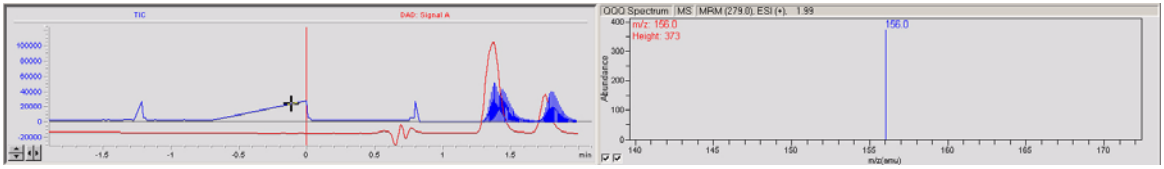
S–Instrument Status; R–Real-time Plot
M–Method Editor; W–Worklist; T-Tune

Instrument Status pane You may have several different LC modules in your LC stack, for example, both a well-plate sampler and micro well-plate sampler. With this pane you can make sure that the correct LC module is configured.

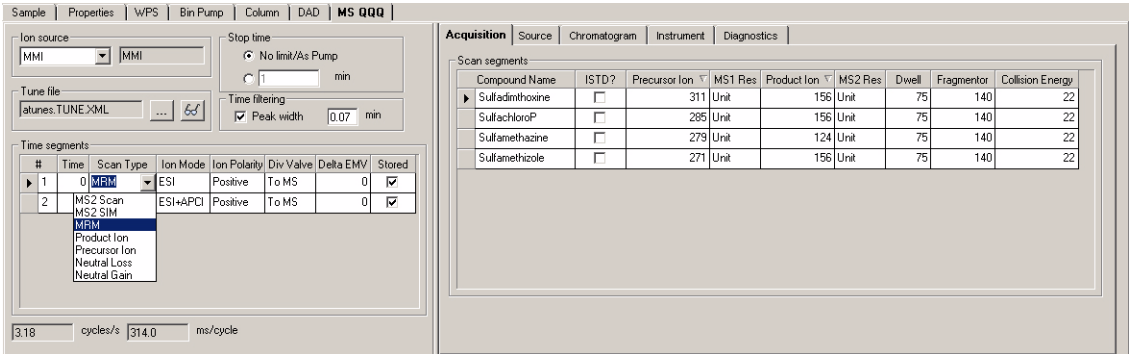
You also set non-method control and configuration parameters for the LC devices and MS device and monitor the status of the device parameters during a run.



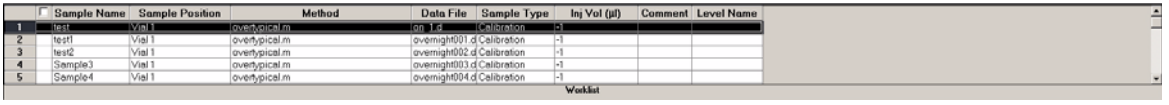
Real-time Plot pane With this pane you monitor the chromatogram and spectral plots in real time.



Method Editor pane With this pane you enter instrument settings for acquisition methods and sample information to run individual samples interactively.



Worklist pane With this pane you enter sample information for individual samples and information for worklist samples. When you run the worklist, the samples are automatically run in the order listed in the worklist.


Sample Name Sample Position Method Data File Sample Type Inj Vol (µl) Comment Level Name
1 test Vial 1 overtypical.m on_1.d Calibration -1
2 test1 Vial 1 overtypical.m overnight001.d Calibration -1
3 test2 Vial 1 overtypical.m overnight002.d Calibration -1
4 Sample3 Vial 1 overtypical.m overnight003.d Calibration -1
5 Sample4 Vial 1 overtypical.m overnight004.d Calibration -1
Worklist

Step 2. Prepare the LC modules

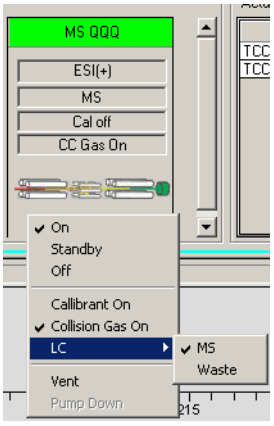
Read and follow the instructions in the online Help for each of the tasks in the checklist described on the following pages.

1 Switch LC stream to Waste.

While you condition or equilibrate the column, you can tune the Triple Quad MS. During this time you do not want pump effluent streaming into the Triple Quad MS, so you switch the direction of the LC stream away from the MS ion source and to waste.

If you have the LC connected to a VWD or DAD, you can still monitor the fluctuations of the VWD or DAD real-time chromatogram before a run.

a Right-click the **MS QQQ** device in the Instrument Status pane.



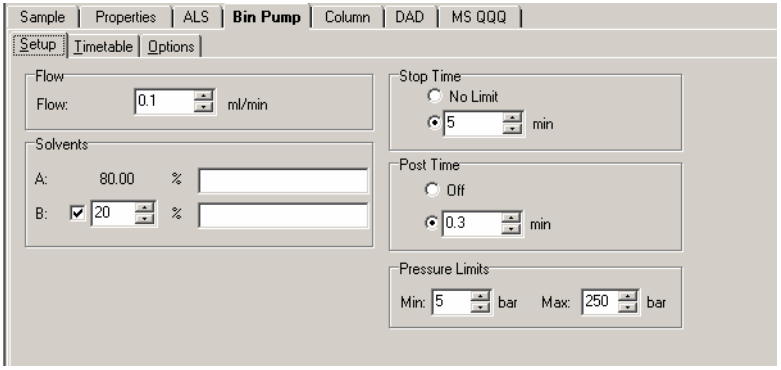
b Click **LC > Waste**.

2 Purge the LC pump.

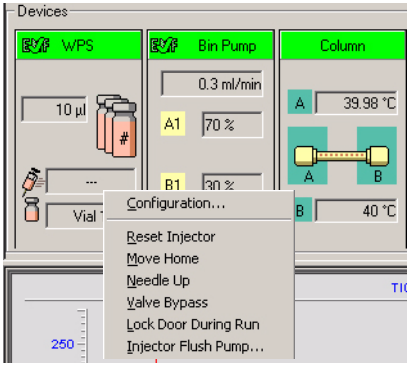
Follow the directions for purging the pump in the *User Guide* for your pump.

3 Set up to condition or equilibrate the column.

a Type LC parameters, and click **Apply** to download them to the LC.



b Right-click an LC module in the Instrument Status pane to change any non-method control parameters, if necessary.

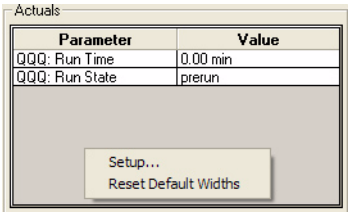


c Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See step 4 and step 5 on page 11.)

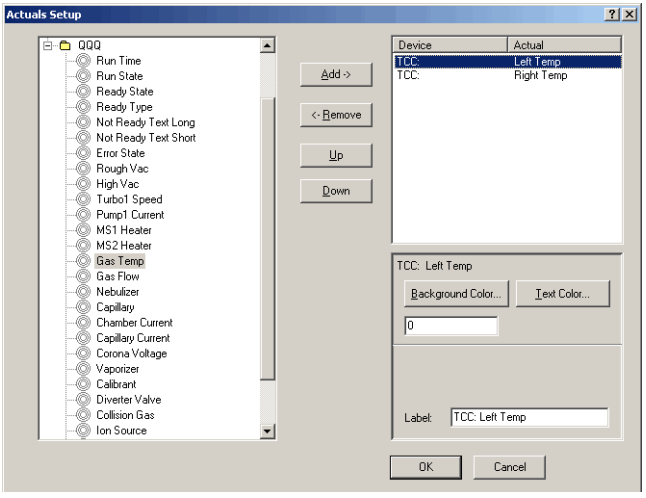
4 Set up to view real-time parameter values (actuals).

As you prepare for a run and during a run, you want to see the actual values of the instrument parameters. You can do this in the Instrument Status pane.

a Right-click the **Actuals** list to see the Setup command.



b Click **Setup** to bring up the list of Actuals available for monitoring.

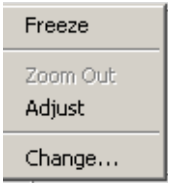


c Add all the parameter values you intend to monitor, and click **OK**.

5 Set up real-time plot displays.

As you condition the column, you set up the displays to monitor the effluent.

- Right-click the chromatogram plot, and click **Change**.

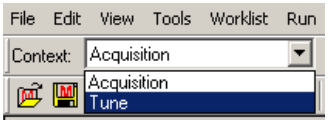


In the Edit Signal Plot dialog box, you can select any type of display signal.

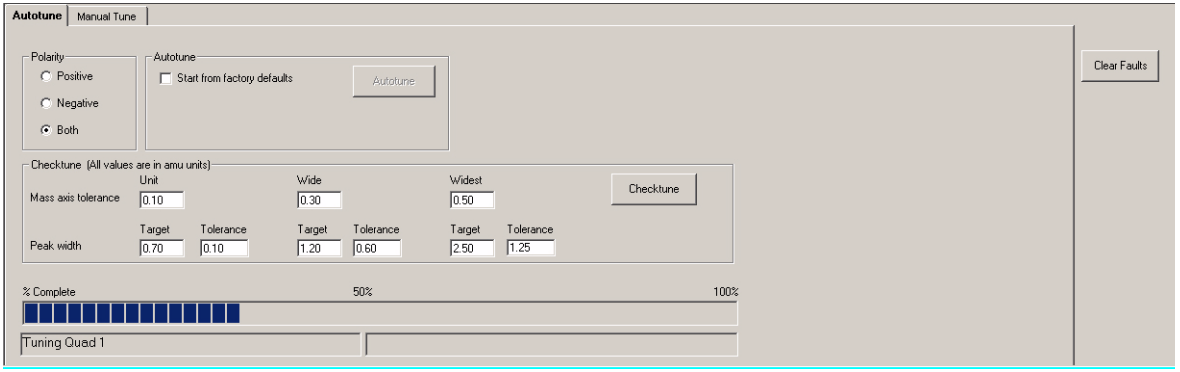
Step 3. Prepare the Triple Quad instrument

Do a Checktune, Autotune or Manual Tune

1 From the **Context** list, click **Tune**.



You can see the Instrument Status pane, the Real-time Plot pane and the Tune pane when you switch to the Tune context. Click the Tune icon in the toolbar if the Tune pane is not visible.



2 Click **Checktune** to evaluate if the MS parameters are within the limits to produce the specified mass accuracy and resolution (2 to 5 minutes).

Do a Checktune regularly.

If Checktune passes, then skip to the next section, "Switch LC stream to MS" on page 14.

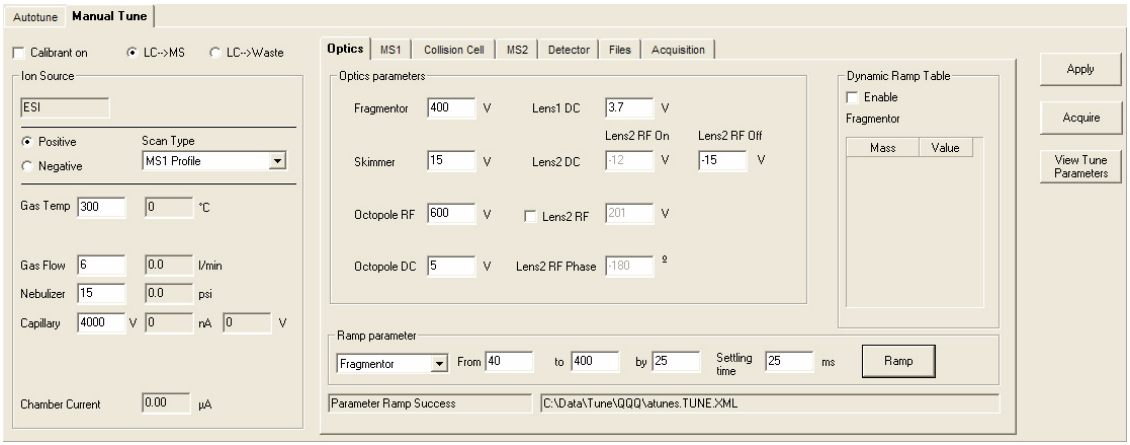
If Checktune fails, then you need to do an Autotune, which is described next.

3 Click **Autotune** to tune the MS automatically (15 to 20 minutes). The system automatically changes different tune parameters to tune the MS. You only do an Autotune when it is necessary.

You only mark the **Start from Factory Defaults** button after an instrument shutdown and restart. Then, if you click **Autotune**, the instrument is tuned starting from the factory defaults (20-25 minutes).

A report is automatically generated after Autotune completes. If Autotune fails, no report is printed.

If Autotune fails or you assess that the Agilent Triple Quad MS needs custom values entered for its tune parameters, you can also do a Manual Tune.



4 From the **Context** list, click **Acquisition**.

Switch LC stream to MS

- After you condition the column and tune the Triple Quad MS, you switch the LC stream from Waste to MS. See "Switch LC stream to Waste." on page 9 for how to do this.

Monitor MS baseline and spectral displays

If you did not monitor the LC baseline with a VWD or DAD, make sure that the Triple Quad baseline is stable and no spectra of interfering intensity appear in the display.

If you did monitor the LC baseline with a VWD or DAD, change back to the default Triple Quad displays.

1 Right-click the chromatogram plot, and click **Change**.
2 Select the MS signal, and click **OK**.

View the system logbook for events and errors

As you prepare the instrument, you may run into an error that you want to troubleshoot. You do this through the System Logbook Viewer.

- Click **Tools > System Logbook Viewer** or click the

icon in the toolbar of the Data Acquisition window, and view the logged events.

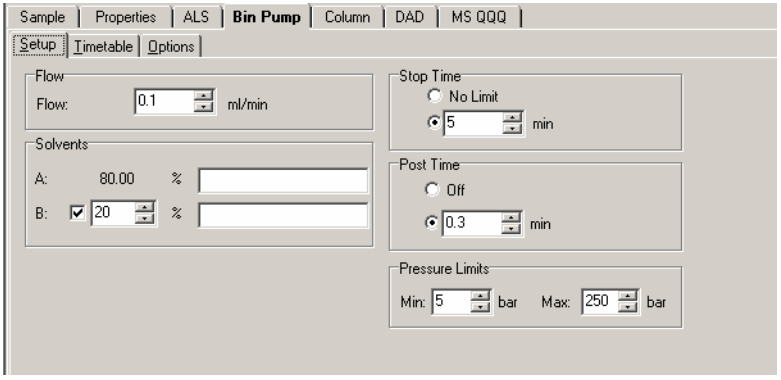
Step 4. Set up and run an acquisition method

Read and follow the instructions in the online Help for each of the tasks described on the following pages.

Also, do Exercise 1 of the Data Acquisition for 6410 *Familiarization Guide* to learn how to set up and run an acquisition method.

1 Set up the method:
   - **a** Type the values and settings for each of the dialog boxes below.
   - **b** (optional) If you want to download the settings to the instrument, click **Apply**.
   - **c** To save the method, click **File > Save As > Method**.
   - **d** Name the method and click **OK**.

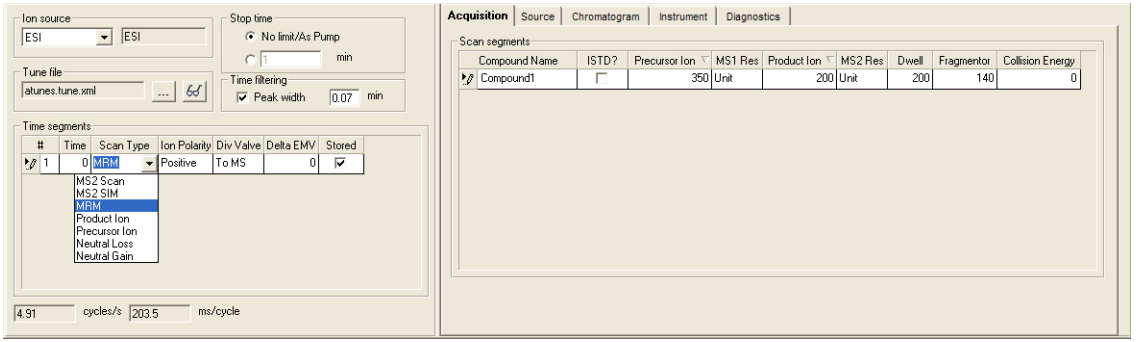
2 Type values for all of the LC modules configured for the instrument.



NOTE Make sure when you type the MS parameters on the next page that the tune file is the one that you want to use with the acquisition.

3 Type Triple Quad MS parameter values.
   - **a** Click the **Scan Type** from the list.

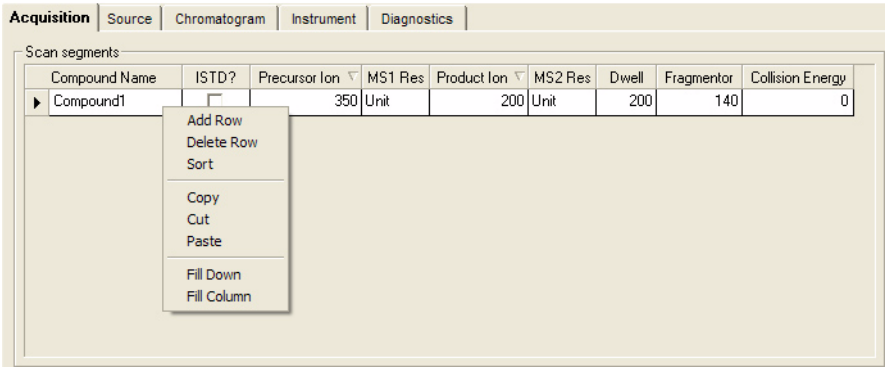
     The parameters available on the right change depending on the Scan Type.
   - **b** Type in any **Acquisition** values you want to change. You can enter multiple Scan segments.



4 Set up to change Triple Quad MS parameters with segments and scans:

a To add a segment, right-click anywhere in the **Scan segments** section to bring up the scan segment menu, and click **Add Row**.

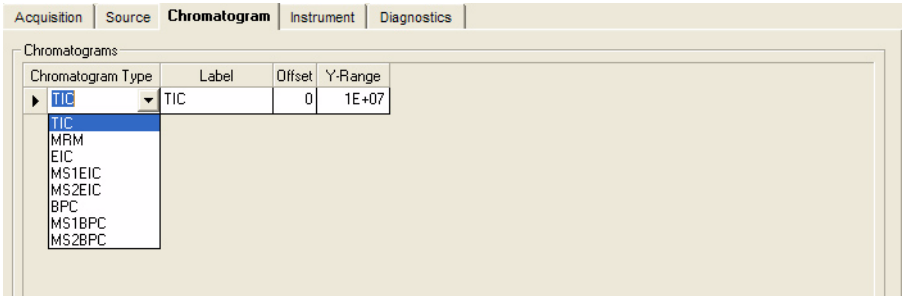
b Type the parameters for each segment.



5 Set up signals for the real-time plot:

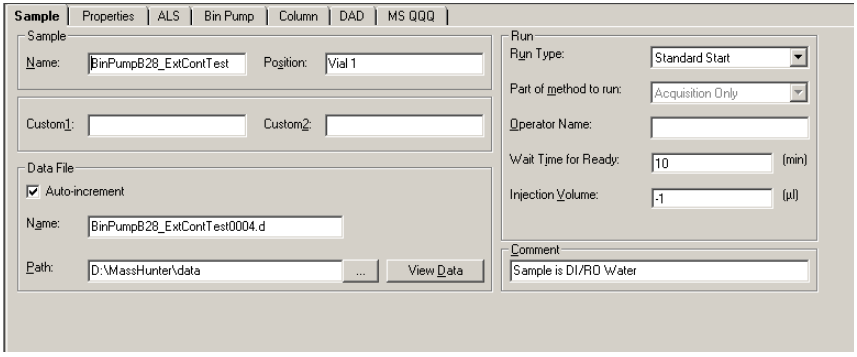
a Click the **Chromatogram** tab.

b Click the **Chromatogram Type**, and type plot values.



6 Set up and run interactive samples:

a Type the sample name, data file name and pathway, and method name.

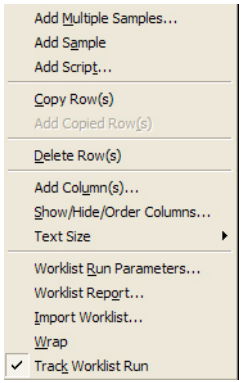


b To start the run, click the single sample icon,

You can run the single sample in either locked or unlocked mode. When the mode is locked, no one can change the method or sample parameters.

7 Set up and run worklists

a Right-click the upper left corner of the worklist.



b Click **Add Multiple Samples.**

c Type all relevant information, and click **OK**.

d To set up the worklist run, right-click the upper left corner, and click **Worklist Run Parameters**.

e Type the paths for the method and data files, and click the tab to specify the sample vial locations (make sure the specific sample tray type has been configured by right-clicking the autosampler device image).

f Specify the locations, and click **OK**.

g To start the run, click the **Multiple Samples** icon, .

You can run the worklist in either locked or unlocked mode. When the mode is locked, no one can change the method or the worklist while the worklist is running.

Step 5. Review results with the Qualitative Analysis program

- Use the Qualitative Analysis program to do these tasks and more:
  - Review results for acquisition method development
  - Select the most appropriate precursor and product ions for MRM analyses
  - Find compounds
  - Identify compounds
  - Do molecular feature extraction

Do the exercises in the ***Qualitative Analysis Familiarization Guide*** to help you learn how to use the Qualitative Analysis program.

Do Exercise 1 of the Data Acquisition for Triple Quad ***Familiarization Guide*** to help you learn how to use the Qualitative Analysis program to develop acquisition methods.

Step 6. Set up and run quantitation

The primary tool for analyzing and reporting Triple Quad LC/MS results is the Quantitative Analysis program.

- Do the exercises in the ***Quantitative Analysis Familiarization Guide*** to learn how to do these operations to quantitate the acquired data files:
  - Set up a batch and a method to automatically quantitate a set of samples
  - Review results by learning how to view and use the Batch-at-a-Glance results screen
  - Identify and use outliers to change the method and requantitate the data using a better calibration curve fit or other more appropriate settings

Also, refer to online Help to learn how to do more operations to analyze your data.

www.agilent.com

In this book

This book contains brief instructions to help you get started with your Agilent Triple Quad LC/MS. This book takes a quick look at using the Data Acquisition program to:

- Prepare the instrument for a run.
- Set up acquisition methods.
- Set up and run worklists.

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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Fourth Edition, December 2007

G3335-90032

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